

BISPHENOL A IS RELEASED FROM USED POLYCARBONATE ANIMAL CAGES INTO WATER AT ROOM TEMPERATURE

**KL Howdeshell, PH Peterman, BM Judy, JA Taylor, CE
Orazio, RL Ruhlen, FS vom Saal, and WV Welshons**
doi:10.1289/ehp.5993 (available at <http://dx.doi.org/>)
Online 5 February 2003



1
2
3
4
5 BISPHENOL A IS RELEASED FROM USED POLYCARBONATE ANIMAL CAGES
6 INTO WATER AT ROOM TEMPERATURE
7

8 KL Howdeshell¹, PH Peterman², BM Judy³, JA Taylor³, CE Orazio², RL Ruhlen¹, FS
9 vom Saal¹, and WV Welshons³.

10 ¹Division of Biological Sciences, University of Missouri, Columbia 65211

11 ²Columbia Environmental Research Center (USGS), Columbia, MO 65201

12 ³Department of Veterinary Biomedical Sciences, University of Missouri, 65211
13
14

15 **Corresponding Author:**

16 Kembra L. Howdeshell, Ph.D.

17 Department of Molecular, Cellular and Developmental Biology

18 830 North University

19 The University of Michigan

20 Ann Arbor, MI 48109-1048

21 Phone: (734) 647-2604

22 Fax: (734) 647-0884

23 Email: kembrah@umich.edu

1 **Running title:** BPA is released from polycarbonate animal cages.

2

3 **Key words:** animal caging, bisphenol A, endocrine disruptor, estrogen, leaching,
4 polycarbonate, and polysulfone.

5

6 **List of abbreviations:**

7 AAALAC - Association for Assessment and Accreditation of Laboratory Animal Care
8 ANCOVA - analysis of covariance
9 BPA - bisphenol A
10 Ctrl - control
11 C - carbon (Figure 3)
12 C - Celsius
13 cm - centimeter
14 CO₂ - carbon dioxide
15 DES - diethylstilbestrol
16 E - 17 β -estradiol
17 Da - Dalton
18 ERIN - estrogen-receptor indicator [mice]
19 GC/MS - gas chromatography/mass spectrometry
20 HBSS - Hank's balanced salts solution
21 HPLC - high pressure liquid chromatography
22 hr - hour
23 LS - least squared [means]
24 LY - keoxifene
25 m - meter
26 MeOH - methanol
27 MEM - minimal essential medium
28 mL - milliliter
29 m/z - mass per charge
30 ng - nanogram
31 O - oxygen
32 P - probability
33 PC - polycarbonate
34 PP - polypropylene
35 PS - polysulfone
36 PND - postnatal day
37 PVC - polyvinyl chloride
38 S - sulfur
39 SAS - Statistical Analyzing System
40 sec - second
41 UV - ultraviolet
42 μ L - microliter
43 μ g - microgram

1 **Outline of section headers:**

2 **INTRODUCTION**

3 **MATERIALS AND METHODS**

4 Animal caging

5 Cage leaching experiment

6 Extraction and gas chromatography (GC/MS) analysis

7 Cell proliferation assay

8 High pressure liquid chromatography (HPLC) analysis

9 Animal housing and conditions

10 Prepubertal mouse uterotrophic assay

11 Statistical analysis

12 **RESULTS**

13 Cage leaching experiment

14 Cell proliferation assay

15 HPLC analysis

16 Prepubertal mouse uterotrophic assay

17 **DISCUSSION**

18 **REFERENCES**

19

20

21

22

23

1 **ABSTRACT**

2 Bisphenol A (BPA) is a monomer with estrogenic activity that is used in the production
3 of food packaging, dental sealants, polycarbonate plastic, and many other products. The
4 monomer has previously been reported to hydrolyze and leach from these products under
5 high heat and alkaline conditions, and the amount of leaching has been reported to
6 increase as a function of use. We examined whether new and used polycarbonate animal
7 cages passively release bioactive levels of BPA into water at room temperature and
8 neutral pH. Purified water was incubated at room temperature in new polycarbonate and
9 polysulfone cages and used (discolored) polycarbonate cages, as well as control (glass
10 and used polypropylene) containers. The resulting water samples were characterized
11 with GC/MS and tested for estrogenic activity using a MCF-7 human breast cancer cell
12 proliferation assay. Significant estrogenic activity, identifiable as BPA by GC/MS (up to
13 310 µg/L), was released from used polycarbonate animal cages. Detectable levels of
14 BPA were released from new polycarbonate cages (up to 0.3 µg/L) as well as new
15 polysulfone cages (1.5 µg/L), while no BPA was detected in water incubated in glass and
16 used polypropylene cages. Finally, BPA exposure as a result of being housed in used
17 polycarbonate cages resulted in a 16% increase in uterine weight in prepubertal female
18 mice relative to females housed in used polypropylene cages, although the difference was
19 not statistically significant. Our findings suggest that laboratory animals maintained in
20 polycarbonate and polysulfone cages are exposed to BPA via leaching, with exposure
21 reaching the highest levels in old cages.

22

23